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Low Sensitivity of Peripheral Blood Smear for Diagnosis of Subclinical Visceral Leishmaniasis in Human Immunodeficiency Virus Type 1-Infected Patients

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The peripheral blood smear is an easy method for the diagnosis of symptomatic visceral leishmaniasis (VL) in human immunodeficiency virus type 1 (HIV-1)-infected patients. However, its efficiency in diagnosing subclinical VL remains unknown. In this study, *Leishmania* amastigotes were seen in blood smears from 1 of 13 HIV-1-positive individuals with subclinical VL. This shows that this procedure is not suitable for subclinical-VL diagnosis.

Visceral leishmaniasis (VL) is a frequent opportunistic disease in human immunodeficiency virus type 1 (HIV-1)-infected patients in Spain (6). It also seems to be emerging as an important infection in the HIV-1-infected population of northern Europe (1). Nearly half of the cases of VL in HIV-seropositive patients are subclinical (7).

Direct examination and culture of bone marrow aspirates are the most common methods of diagnosing VL in HIV-1-infected patients. These techniques require invasive procedures, which is why they are difficult to use in large epidemiological surveys and difficult to repeat for follow-up of patients with disease. Thus, an easy and noninvasive method would be valuable in this field. Serology and leishmania skin tests show low sensitivities for HIV-1-infected patients (6). The direct visualization of *Leishmania* amastigotes in peripheral blood smears is an easy method for the diagnosis of symptomatic VL in this population, with a sensitivity of about 50% (5). This seems to be due to a high level of parasitemia in these patients (3, 5). However, to our knowledge, there is no data about the sensitivity of this procedure for patients with HIV-1 infection and subclinical VL.

The objective of the present study was to determine the sensitivity of direct examination of peripheral blood smears for detection of *Leishmania infantum* amastigotes in asymptomatic HIV-infected individuals. In addition, this sensitivity was compared with that for symptomatic VL patients.

In order to assess the prevalence of *Leishmania*-HIV coinfection, all 346 HIV-1-infected patients without severe clotting disorders who were treated at our unit between January 1993 and April 1997 were invited to participate in a cross-sectional study. A total of 302 (87%) patients agreed to participate. A sternal-bone marrow aspiration (BMA) was performed for all patients. Peripheral blood smears were taken at the time of the BMA for all patients included since August 1993.

The study was designed and performed according to the Helsinki Declaration and approved by the Hospital Ethics Committee; all patients gave their written informed consent to participate.

Thin and thick smears were made with peripheral blood and BMA samples. They were stained with Giemsa stain and examined at a ×1,000 magnification for at least 20 min by an experienced parasitologist.

Symptomatic VL was defined as described elsewhere (7). Briefly, a case of VL was considered subclinical if fever, splenomegaly, and a hemoglobin level of <9 g/dl were absent.

Leishmania amastigotes were found in 35 patients for whom BMA was performed (11.6%). Nineteen (54.3%) had symptomatic infections, and 16 (45.7%) had subclinical infections. Peripheral blood smears were available from 15 of the 19 symptomatic patients and from 13 of the 16 patients with subclinical infections. Amastigotes were identified in peripheral blood smears from 10 of the 15 symptomatic VL patients (66.7%), while only 1 out of the 13 blood smears taken from patients with subclinical VL was positive (7.7%) ($P = 0.001$ by Fisher's test).

For HIV-1-infected patients, the sensitivity of direct examination of peripheral blood smears is lower for subclinical-VL patients than for symptomatic VL patients. This could be due to a lower parasite burden in subjects with subclinical VL. Because of this, these patients would be less parasitemic and the possibility of visualizing amastigotes in peripheral blood preparations would be much smaller. A matter not yet clarified is whether low parasite burdens in subclinical-VL patients are due to adequate immune responses against *Leishmania* or to infection with less virulent strains.

Microscopic examination of peripheral blood smears is not a good procedure for the diagnosis of subclinical VL in HIV-1-infected individuals. Therefore, more sensitive methods that are quicker and easier than the investigation of *Leishmania* in tissue are urgently needed. Techniques such as PCR with DNA from peripheral blood mononuclear cells (8) and examination of buffy coats (4) may be more sensitive than direct visualization of peripheral blood smears. These methods could allow us to perform large epidemiological surveys among HIV-1-infected individuals. On the other hand, VL could act as a cofactor in the progression of the HIV-1 disease (2). Whether or not subclinical VL silently accelerates the course of HIV-1, these methods would also be necessary for early diagnosis of VL in HIV-1-infected patients.

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